PB87-105532

Algal-Induced Decay and Formation of Hydrogen Peroxide in Water Its Possible Role in Oxidation of Anilines by Algae

(U.S.) Environmental Research Lab., Athens, GA

Oct 86

U.S. Department of Commerce
National Technical Information Service
NTIS

PB87-105532

EPA/600/D-86/227
October 1986

ALGAL-INDUCED DECAY AND FORMATION OF HYDROGEN PEROXIDE IN WATER: ITS POSSIBLE ROLE IN OXIDATION OF ANILINES BY ALGAE

by

R.G. Zepp, Y.I. Skurlatov*, and J.T. Pierce

Environmental Research Laboratory
U.S. Environmental Protection Agency
Athens, Georgia 30613
*Institute of Chemical Physics
Academy of Sciences
Moscow, USSR

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30613

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-86/227		PB87 105532/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
ALGAL-INDUCED DECAY AND FORMATION OF HYDROGEN PEROXIDE IN WATER: ITS POSSIBLE ROLE IN OXIDATION OF ANILINES BY ALGAE	October 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
R.G. Zepp, Y.I. Skurlatov*, and J.T. Pierce	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, GA 30613 *Institute of Chemical Physics, Academy of Sciences, Moscow, USSR	ACUL1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory - Athens, GA Office of Research and Development U.S. Environmental Protection Agency Athens, GA 30613	14. SPONSORING AGENCY CODE EPA/600/01

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Studies of the rates of decomposition and photoproduction of hydrogen peroxide (H_2O_2) by several green and blue-green algae in water are reported. Results suggest that algae have an important influence on the environmental concentration of H_2O_2, a widely distributed oxidant in natural waters. The algal-catalyzed decomposition of H_2O_2 in the dark was found to be second-order overall, first-order with respect to H_2O_2, and first-order with respect to algal biomass. Exposure of algal suspensions to sunlight resulted in a build up of H_2O_2, indicating that algae can photoproduce as well as decompose this oxidant. Kinetic results for the algal-induced photooxidation of substituted anilines are presented and a mechanism involving the intermediacy of H_2O_2 is discussed.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	13
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Algal-induced Decay and Formation of Hydrogen Peroxide in Water: Its Possible Role in Oxidation of Anilines by Algae

R.G. Zepp, Y.I. Skurlatov[1], and J.T. Pierce

Environmental Research Laboratory
U.S. Environmental Protection Agency
Athens, Georgia 30613

Studies of the rates of decomposition and photoproduction of hydrogen peroxide (H_2O_2) by several green and blue-green algae in water are reported. Results suggest that algae have an important influence on the environmental concentration of H_2O_2, a widely distributed oxidant in natural waters. The algal-catalyzed decomposition of H_2O_2 in the dark was found to be second-order overall, first-order with respect to H_2O_2, and first-order with respect to algal biomass. Exposure of algal suspensions to sunlight resulted in a buildup of H_2O_2, indicating that algae can photoproduce as well as decompose this oxidant. Kinetic results for the algal-induced photooxidation of substituted anilines are presented and a mechanism involving the intermediacy of H_2O_2 is discussed.

Recent investigations have shown that hydrogen peroxide (H_2O_2) is a common constituent of the hydrosphere, including clouds, rainwater, freshwater, and seawater (1-3). In Figure 1, we have summarized several of the processes likely to be important in the formation and decay of H_2O_2 in natural water bodies.

Both field and laboratory studies indicate that the major pathway for production of H_2O_2 in natural waters is photochemical (2-3), although it also is introduced to water bodies through rain and biological processes. The photochemical formation is most likely mediated by electron transfer to dioxygen to form superoxide ion, an oxidant that dismutes to form H_2O_2. The conjugate acid of superoxide ion, the hydroperoxyl radical, also rapidly disproportionates to form hydrogen peroxide (4).

---

[1]Current address: Institute of Chemical Physics, Academy of Sciences of the Union of Soviet Socialist Republics, Moscow, USSR.

Several pathways can account for the decomposition of hydrogen peroxide in natural waters. Some of these decay processes not only remove H_2O_2, but also result in the oxidation of chemicals, possibly including various pollutants, that are present in natural waters. These processes include direct oxidation (5), peroxidase-catalyzed oxidation (6), and free radical oxidation initiated by photochemical or metal-catalyzed decomposition (7). Little is known about the significance and rates of these various processes under environmental conditions, but they have all been shown to occur rapidly with certain organic substrates in the laboratory.

Many previous studies have shown that the light-induced transformation of certain pollutants is greatly accelerated in natural waters compared to distilled water. Oxidations via photoproduced H_2O_2 may account for the results of some of these investigations. In this paper, we report results that quantify the rates of photoproduction and decomposition of H_2O_2 by several pure cultures of algae in water. Results also are presented which indicate that H_2O_2 or a precursor thereof may be involved in the algal-induced photooxidation of anilines.

Experimental

Materials. Analytical grade m-toluidine and p-butylaniline were obtained commercially from ChemService and used as received. Aniline (Aldrich) was purified by treating an aqueous solution with activated charcoal (0.1% by weight). The charcoal was removed by filtration through a 0.2 μm Millipore filter. The filtrate was extracted by ether and, after drying over anhydrous sodium sulfate, the layer was evaporated under vacuum. The aniline finally was distilled under vacuum. Horseradish peroxidase (HRP) (activity 152 purpurogallin units/mg), catalase (from bovine liver, activity 17,600 units/mg protein) and leuco malachite green (LMG) [4,4'benzylidene-bis (N,N-dimethylaniline] were obtained from Sigma Chemicals. The LMG was purified by first washing a benzene solution (0.1 g/ml) with 5% aqueous sodium hydroxide. The benzene layer was separated, dried over anhydrous sodium sulfate, then evaporated to dryness. The resulting residue was recrystallized twice from hexane.

The algae used in the studies were either obtained on slants from the Starr collection (8) at the University of Texas, Austin, Texas, or from a collection of Dr. Gary Kochert, Botany Department, University of Georgia, Athens, Georgia. The bacteria-free algae from the Starr collection were handled and grown under aseptic conditions. UTEX 76, UTEX 26, UTEX 89, Chlamydomonas sp., Chlorococcum hypnosporum, Anabaena variabilis, and Chlorogonium sp. were grown in medium and under conditions specified by Zepp and Schlotzhauer (9). UTEX 625 and UTEX 1444 were grown at 39°C in the media that is described by Kratz and Myers (10) as modified by Van Baalen (11) using an 8:16 hr light-dark cycle.

Equipment. The algae were grown in a New Brunswick Scientific Co. controlled environment incubator shaker. Kinetic studies were

conducted using algae suspensions that were agitated on a New Brunswick G-10 gyrotary shaker.

A Perkin Elmer Lambda 3 uv-visible spectrophotometer was used in the peroxide assays. The algae suspensions were filtered using Micromate 5-cm^3 glass syringes equipped with Millipore filter holders containing Whatman GF/C filter pads.

Aniline solutions were analyzed using a Micromeritics Model 7170 high pressure liquid chromatograph equipped with a column packed with ODS-3; mixtures of acetonitrile and water were used as mobile phase.

Kinetic Procedures. The algae culture suspension (100ml) was placed in a sterile 200-ml Erlenmeyer flask. The flask was covered with aluminum foil to block out light. The temperature of the reaction was kept at 18°C throughout the experiment. The kinetic run was started by adding sufficient H_2O_2 to result in an initial concentration of 5.0 μM.

The reaction mixture was then placed on the shaker at a shaker speed of 100 rpm. After one minute of shaking, a 4.5-ml aliquot of the solution was filtered and mixed with 0.5 ml of a peroxidase/LMG assay solution (12 μM LMG, 40 mg/L peroxidase in pH 4.0 acetate buffer). A blue color formed immediately when the H_2O_2 and the assay solution were mixed. The sample was then allowed at least 20 minutes to develop completely. More samples were taken at intervals throughout the run until about two halflives were complete.

Using the spectrophotometer, the absorbance of the samples at 617 nm was determined versus distilled water. The absorbance at 750 nm also was determined and subtracted from the 617 nm absorbance to yield a corrected absorbance. A first-order rate constant for the corrected absorbance versus time then was calculated using an HP41C calculator programmed to fit the data by least squares analysis.

After completion of the kinetic run, 30 ml of the remaining algae suspension then was analyzed for its chlorophyll a content, C_a (12). Experiments on the photoproduction of H_2O_2 and the algal-induced oxidation of anilines were conducted in full sunlight as described by Zepp and Schlotzhauer (9) using a p-nitroanisole actinometer (13) to measure light intensity.

Effects of H_2O_2 upon the oxidation of aniline in dark algal cultures were studied. To a suspension (100 ml) of the algae (C_a, 2000-5000 mg chl a/m^3) in phosphate buffer containing 5.0 μM aniline was added sufficient H_2O_2 to make the initial concentration 20 μM. The resulting mixture was incubated on the shaker for three half-lives of the H_2O_2, then a portion (2.0 ml) was removed, filtered and analyzed for aniline and H_2O_2. This procedure was repeated five times. No reaction (<10%) of the aniline was observed after five cycles, indicating that less than 0.005 moles of aniline reacted per mole of decomposed H_2O_2. In a control experiment with

HRP (1 mg/L) substituted for the algae, the aniline was completely oxidized with one cycle of the H_2O_2 decomposition.

Results and Discussion

Kinetic Studies of H_2O_2 Decay. In the presence of all the types of algae studied, the decomposition of hydrogen peroxide was first-order with respect to peroxide. As the typical results in Figure 2 illustrate, the catalysis was heterogeneous; catalysts released by the algae into bulk solution were not involved.

Other studies involving variation in algal concentration indicated that the decay rate of H_2O_2 also was first-order with respect to chlorophyll *a* content, C_a. The complete rate expression was second-order overall:

$$\text{Rate} = k_1 [H_2O_2] = k_{bio} C_a [H_2O_2] \qquad (1)$$

Plots of pseudo-first-order rate constants, k_1, versus algal biomass expressed as micrograms chlorophyll *a*/L were linear (Figure 3).

Second-order rate constants for catalysis of H_2O_2 decay by five freshwater green algae and four cyanobacteria are reported in Table I. Most of the microorganisms examined were quite efficient at removing H_2O_2, indicating that algal-induced decomposition is likely a major pathway for its decay in freshwaters. For example, using the median value of 0.0044 m^3 (mg chl *a*)$^{-1}h^{-1}$, the half-life for H_2O_2 in the euphotic zone of a eutrophic lake (C_a = 100 mg/m^3) is estimated to be about 1.5 hours.

Table I. Kinetic Data for Algal-catalyzed Decay of Hydrogen Peroxide -- 18°C in Dark

Alga	$10^3 k_{bio}$ m^3(mg chl a)$^{-1}h^{-1}$ [a]
Cyanobacteria	
Nostoc muscorum	8.8 ± 2.0
UTEX 625 (*Synechococcus leopoliensis*)	4.3 ± 1.1
UTEX 625 - after 4 hours of sunlight exposure	3.2 ± 0.8
UTEX 1444 (*Anaebaena flos-aquae*)	2.2 ± 0.5
Anabaena variabilis	7.7 ± 1.2
Green algae	
UTEX 76 (*Scenedesmus quadricauda*)	4.4 ± 1.1
UTEX 89 (*Chlamydomonas reinhardtii*)	0.18 ± 0.04
Chlorococcum hypnosporum	6.3 ± 0.9
Chlamydomonas sp.	1.2 ± 0.2
Chlamydomonas sp. - after 5 hours of sunlight exposure	0.1 ± 0.02
Chlorogonium sp.	5.5 ± 1.3

[a] Mean (± s.d. for three kinetic runs)

Rates of H_2O_2 Photoproduction. Exposure of the Chlamydomonas sp. cultures to solar radiation resulted in a buildup of H_2O_2 (Figure 4). The rate of formation increased with increasing light exposure and with increasing algae concentration. Moreover, the ability of the Chlamydomonas to catalyze the decay of H_2O_2 decreased sharply with increasing irradiation (Table I). Sunlight exposure also reduced the H_2O_2-decomposing ability of the cyanobacteria, UTEX 625, but the effect was not nearly as pronounced as with Chlamydomonas (Table I). These results indicate that the degree of H_2O_2 buildup may be related in part to photodegradation of peroxide-decomposing enzymes.

Rates of photoproduction of H_2O_2 by several algae are compared in Table II. The tabulated values correspond to averages observed during a 5-hour exposure to midday sunlight at Athens, Georgia. For unknown reasons, the Nostoc muscorum produced H_2O_2 much less efficiently. The lower efficiency may in part be ascribed to the much larger size (and lower surface area per biomass) of the Nostoc cells (about 50 μm in diameter) compared to the other algae studied (<10 μm).

Table II. Kinetic Data for Photoproduction of Hydrogen Peroxide by Various Algae in Sunlight

Alga	10^{-3} C_a, mg chl a/m^3	Average Rate of Peroxide Production ($M\ h^{-1} \times 10^6$)[a,b]
Chlamydomonas sp.	0.9	0.8
Chlamydomonas sp.	0.097	0.15
Chlorogonium sp.	1.0	0.6
Anaebaena variabilis	0.9	1.7
Nostoc muscorum	1.0	0.04

[a]Peroxide completely decomposed by brief treatment (1 minute) with catalase. [b]Midday, near-surface sunlight intensity, spring, latitude 34°N.

Algal-induced Oxidation of Anilines. Kinetic studies indicated that several anilines were oxidized when exposed to sunlight in suspensions of Chlamydomonas sp., a green algae (Figure 5). In previous studies, we have presented evidence that this oxidation is not metabolic (9). Aniline and its alkyl-substituted derivatives were found to be unreactive in the supernatant from centrifuged algal suspensions, indicating that the photocatalysis was heterogeneous. Variation of the alkyl substituent on the aromatic ring of aniline had a pronounced effect on the rate of the reaction (Figure 5). Relative rate constants for the reaction correlated with octanol-water partition coefficients for the anilines (Table III). This hydrophobic effect supports a reaction mechanism involving partitioning of the substrate on the algal cells, as previously postulated by Zepp and Schlotzhauer (9) (Figure 6).

The above discussed results suggested a possible role of hydrogen peroxide in the aniline photooxidations. In support of

Table III. Comparison of Rate Constants and K_{ow}'s for Alkyl-Substituted Anilines

Compound	log K_{ow} [a]	In Algae Suspension log k_{rel} [b]
Aniline	0.98	-0.73
m-Toluidine	1.40	-0.21
p-n-Butylaniline	2.6 [c]	0.91

[a]Ref. 15; [b]Ratio of rate constant in sunlight to rate constant for p-nitroanisole actinometer (conditions 1 M aniline; 240 mg chl a/m^3 Chlamydomonas sp.; pH 7.0 in phosphate buffer; [c]computed value.

this concept, aniline strongly quenched the photoproduction of H_2O_2 by Chlamydomonas sp. (Figure 4), possibly indicating that the peroxide or a precursor thereof was diverted by reaction with the substrate on the cell. The idea was supported further by earlier studies (14) that showed that anilines are particularly susceptible to oxidations catalyzed by peroxidases. When H_2O_2 was added repetitively to dark algal suspensions (Chlamydomonas, Nostoc, Anabaena) containing anilines, however, no aniline oxidation occurred when the peroxide decomposed. Aniline was rapidly oxidized under the same conditions with added peroxidase. These results indicate that other substances, probably catalase enzymes (16), were responsible for the decomposition of H_2O_2 by the algae included in the present study. The inhibition of photoproduction of H_2O_2 by aniline must be attributed to physical or chemical quenching of a precursor to the H_2O_2 or possibly to a light-induced reaction between H_2O_2 and aniline on the surface of the algal cell (Figure 6). A detailed examination of the nature of this oxidation was beyond the scope of this study.

Conclusions

(1) Green and blue-green algae are capable of efficiently catalyzing the removal of trace hydrogen peroxide from water. A second-order rate expression describes the removal. The median second-order rate constant for nine algae was about $4 \times 10^{-3} m^3$ (mg/chl a)$^{-1} h^{-1}$.

(2) Exposure of algae to sunlight results in photogeneration of hydrogen peroxide. This finding indicates that the microbiota contribute to the photoproduction of peroxide in natural waters, although further research is required to establish the generality of this photoprocess.

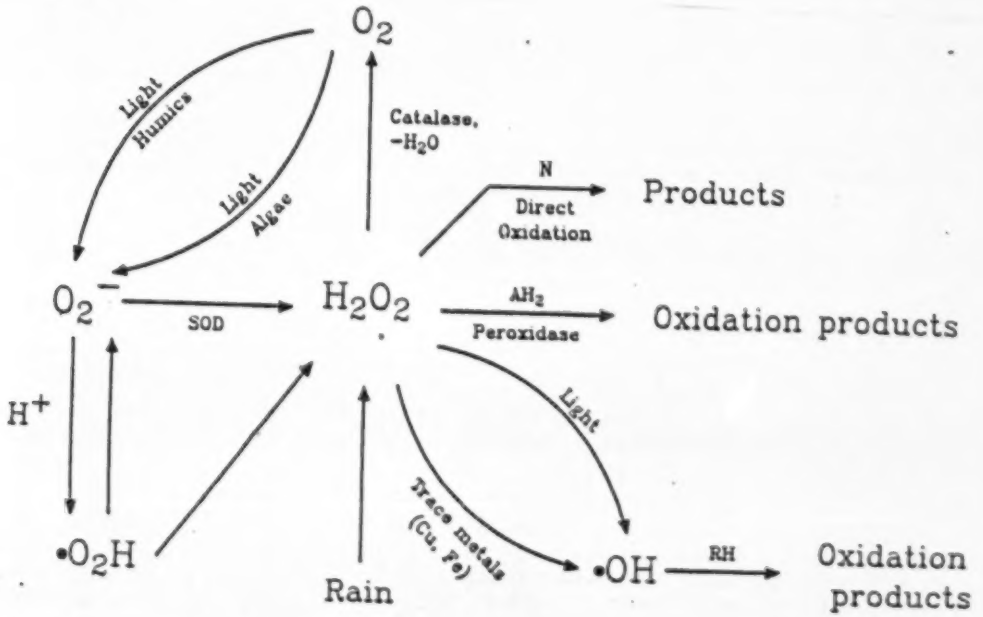
(3) A green algae photocatalyzed the oxidation of anilines in a reaction that exhibited a pronounced hydrophobic effect. Anilines also inhibited the photoproduction of H_2O_2 by Chlamydomonas indicating that photochemically produced peroxide or a precursor thereof plays a key role in the oxidation.

Acknowledgments

A portion of this work was performed under the Bilateral Agreement of Environmental Protection, Project 02.03-31 "Forms and Mechanisms by Which Pesticides and Chemicals are Transported", between the United States and the Union of Soviet Socialist Republics. The technical assistance of J. Mims and C. Bakke is acknowledged as is the preparation of the figures by B. Bartell, R. Moon and P. Winter.

Literature Cited

1. Penkett, S.A.; Jones, B.M.R.; Brice, K.A.; Eggleton, A.E.J. Atmos. Environ. 1979, 13, 123.
2. Cooper, W.J.; Zika, R.G. Science 1983, 220, 711.
3. Draper, W.; Crosby, D.G. Arch. Environ. Cont. Toxicol. 1983, 12, 121.
4. Bielski, B.H.J.; Cabelli, D.E.; Arudi, R.L.; Ross, A.B. J. Phys. Chem. Ref. Data 1985, 14, 1041-1100.
5. Leung, P-S.K.; Hoffmann, M.R. J. Phys. Chem. 1985, 89, 5267.
6. Dunford, H.B.; Stillman, J.S. Coord. Chem. Rev. 1976, 19, 187-251.
7. Draper, W.M.; Crosby, D.G. J. Agr. Food Chem. 1984, 32, 231.
8. Starr, R.C. J. Phycol. 1978, 14, 47-100.
9. Zepp, R.G.; Schlotzhauer, P.F. Environ. Sci. Technol. 1983, 17, 462-468.
10. Kratz, W.A.; Myers, J. Am. J. Bot. 1955, 42, 282-287.
11. van Baalen, C.; Marler, J.E. Nature 1966, 211, 951.
12. "Standard Methods for the Examination of Water and Wastewater"; 13th Ed.; American Public Health Association: New York, 1971; pp. 746-747.
13. Dulin, D.; Mill, T. Environ. Sci. Technol. 1982, 16, 815.
14. Job, D.; Dunford, H.B. Eur. J. Photochem. 1976, 66, 607.
15. Leo, A.; Hansch, C.; Elkins, D. Chem. Rev. 1971, 71, 525.
16. Saunders, B.C.; Holmes-Seidle, A.G.; Stark, B.P. "Peroxidase"; Butterworths: London, 1964.



Definitions: SOD – Superoxide dismutase in biota
N – Nucleophile that reacts with H_2O_2
AH_2 – Substrate capable of oxidation by peroxidases, H_2O_2 (e.g. phenols, anilines)
RH – Organic chemical

Figure 1. Processes that contribute to the formation and decay of hydrogen peroxide in aquatic environments.

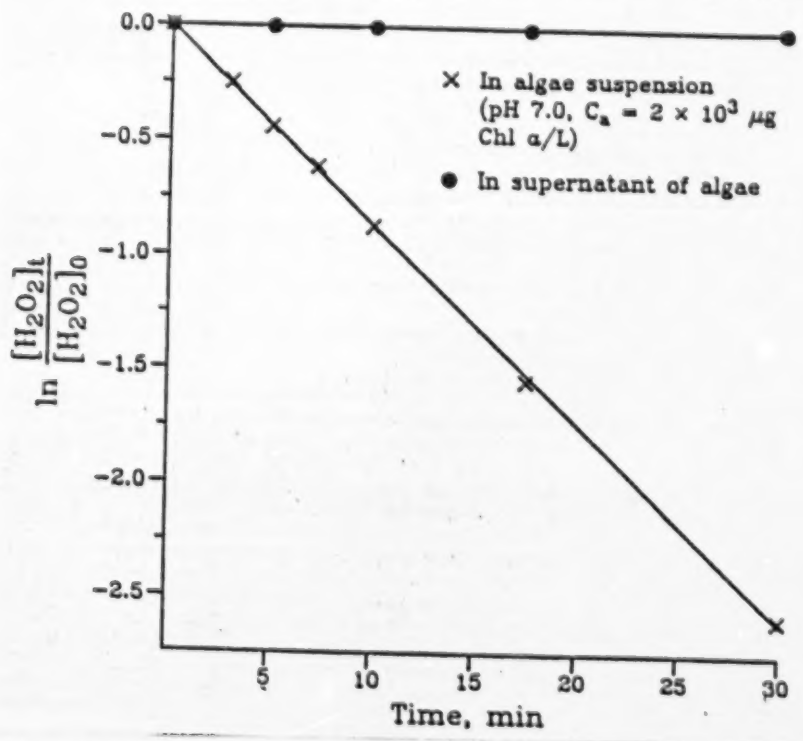


Figure 2. Kinetic data for the decay of hydrogen peroxide in suspension of UTEX 1444.

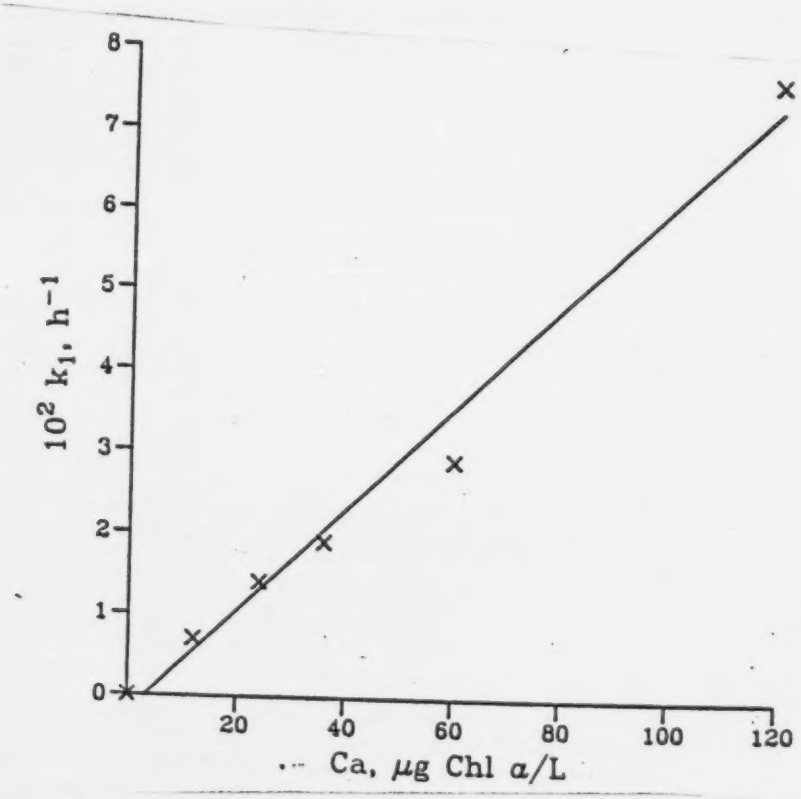


Figure 3. Dependence of first-order rate constant for decay of hydrogen peroxide on concentration of Chlamydomonas sp.

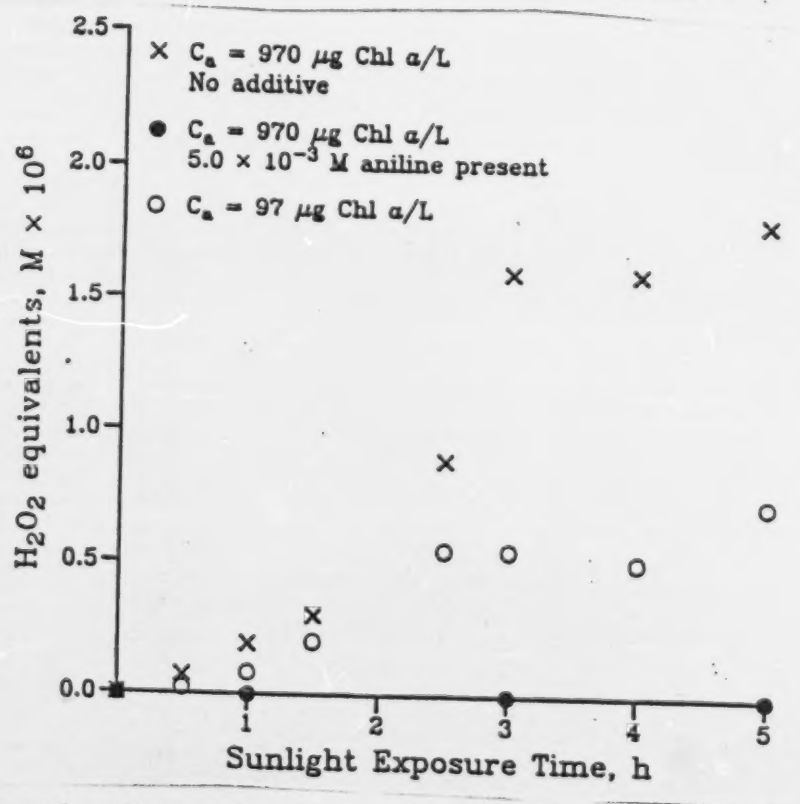


Figure 4. Photoproduction of hydrogen peroxide in suspensions of Chlamydomonas sp. exposed to sunlight.

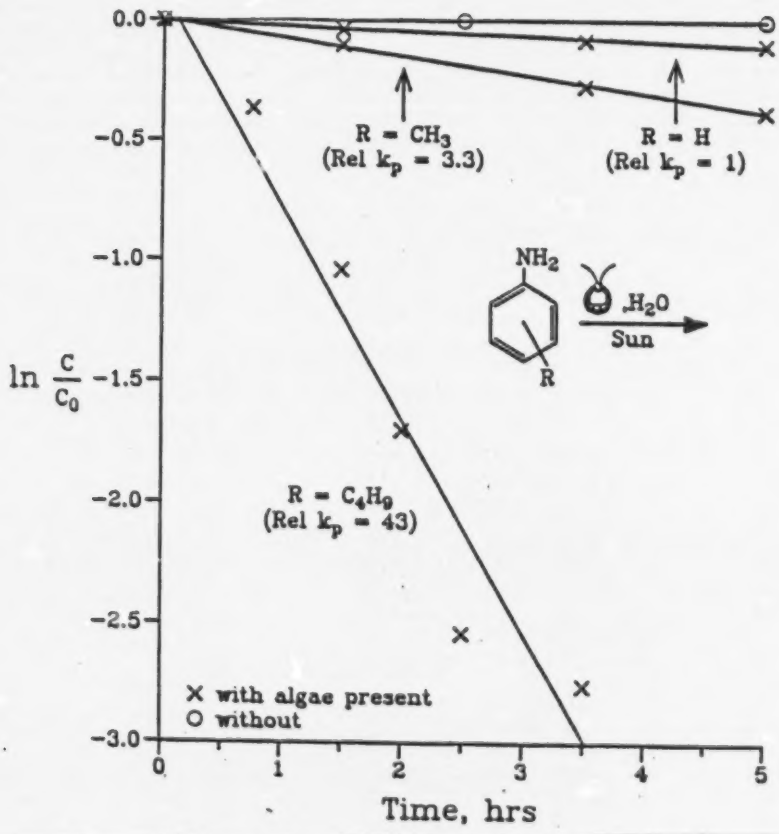


Figure 5. Kinetic plots for oxidation of anilines in suspension of Chlamydomonas sp. exposed to sunlight.

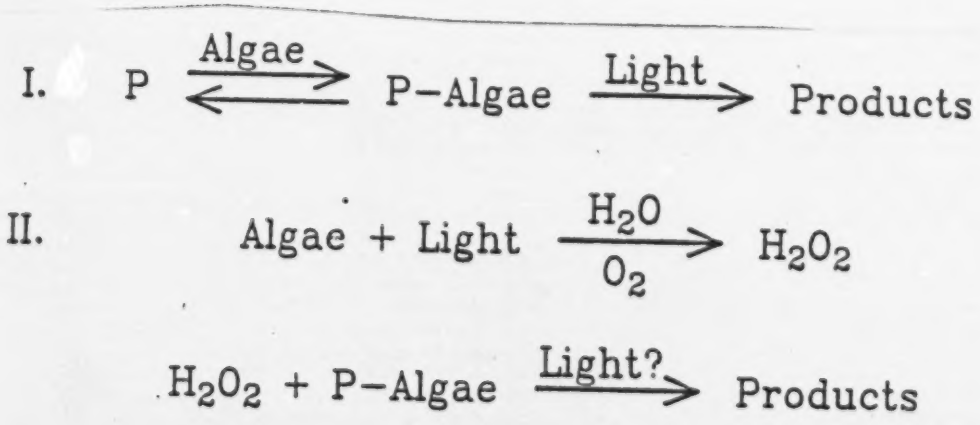


Figure 6. Possible pathways for the algal-induced oxidation of anilines.